

## CLAIMS

We claim:

1. A method, comprising:
  - 5 a) providing
    - i) a sample comprising a plurality of polypeptides;
    - ii) a first separation device configured for separation of said polypeptides in said sample based on charge;
    - 10 iii) a second separation device configured for separation of said polypeptides in said sample based on hydrophobicity; and
    - iv) a third separation device configured for separation of said polypeptides in said sample based on size; and
  - b) separating said sample with said first separation device to generate a charge separated protein sample, wherein said charge separated sample comprises a plurality of fractions;
  - 15 c) separating said charge separated sample with said second separation device to generate a charge and hydrophobicity separated sample, wherein said charge and hydrophobicity separated sample comprises a plurality of fractions; and
  - d) separating said charge and hydrophobicity separated sample with said 20 third separation device to generate a charge, hydrophobicity, and size separated sample, wherein said charge, hydrophobicity and size separated sample comprises a plurality of fractions.
2. The method of claim 1, wherein said first separation device is configured for 25 performing a separation technique selected from the group consisting of isoelectric focusing gel electrophoresis, free-flow electrophoresis, rotofor electrophoresis and ion exchange chromatography.
3. The method of claim 1, wherein said second separation device is configured 30 for performing a separation technique selected from the group consisting of reversed-phase chromatography and hydrophobic interaction chromatography.

4. The method of claim 1, wherein said third separation device is configured for performing a separation technique selected from the group consisting of SDS-gel electrophoresis, size exclusion chromatography, and capillary electrophoresis.

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5. The method of claim 1, further comprising the step of detecting polypeptides in said fractions of said charge, hydrophobicity, and size separated sample.

6. The method of claim 5, wherein said detecting comprises a detection method selected from the group consisting of UV/VS spectrophotometry, fluorescence spectrophotometry, and mass spectrometry.

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7. The method of claim 6, wherein said mass spectroscopy is selected from the group consisting of MALDI-TOF-MS, ESI oa TOF, ion trap mass spectrometry, ion trap/time-of-flight mass spectrometry; quadrupole mass spectrometry, triple quadrupole mass spectrometry, Fourier Transform (ICR) mass spectrometry, and magnetic sector mass spectrometry.

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8. The method of claim 1, further comprising the step of attaching said plurality of fractions of said charge, hydrophobicity, and size separated sample to a solid support.

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9. The method of claim 8, wherein said plurality of fractions are arrayed on said solid support.

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10. The method of claim 9, further comprising the step of performing a functional assay on said arrayed plurality of fractions.

11. The method of claim 10, wherein said functional assay comprises an antibody binding assay.

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12. The method of claim 1, wherein said plurality of polypeptide comprise a proteome.

13. The method of claim 1, further providing a second sample comprising a plurality of polypeptides.

14. The method of claim 13, wherein said sample comprises a proteome of a non-cancerous cell and said second sample comprises a proteome of a cancerous cell.

15. The method of claim 14, further comprising the step of comparing said charge, hydrophobicity, and size separated sample to a charge, hydrophobicity, and size separated second sample.

16. A protein separation apparatus, comprising a first separation device, wherein said first separation device is a protein charge separation device; a second separation device, wherein said second device is a protein hydropobicity separation device; and a third separation device, wherein said third separation device is a protein size separation device.

17. The apparatus of claim 16, wherein said first separation device is selected from the group consisting of a isoelectric focusing gel electrophoresis device, a free-flow electrophoresis device, a rotofor electrophoresis device, and an ion exchange chromatography device.

18. The apparatus of claim 16, wherein said second separation device is selected from the group consisting of a reversed-phase chromatography device and a hydrophobic interaction chromatography device.

19. The apparatus of claim 16, wherein said third separation device is selected from the group consisting of an SDS-gel electrophoresis device, a size exclusion chromatography device, and a capillary electrophoresis device.

20. The apparatus of claim 16, further comprising a detection device.

21. The apparatus of claim 20, wherein said detection device is selected from the group consisting of a UV/VS spectrophotometer, a fluorescence spectrophotometer, and a  
5 mass spectrometer.

22. The apparatus of claim 21, wherein said mass spectrometer is selected from the group consisting of a MALDI-TOF-MS, a ESI oa TOF, an ion trap mass spectrometer, an ion trap/time-of-flight mass spectrometer; a quadrupole mass spectrometer, a triple  
10 quadrupole mass spectrometer, a Fourier Transform (ICR) mass spectrometer, and a magnetic sector mass spectrometer.

23. A system comprising a protein separation apparatus, said apparatus comprising a first separation device, wherein said first separation device is a protein charge  
15 separation device; a second separation device, wherein said second device is a protein hydrophobicity separation device; and a third separation device, wherein said third separation device is a protein size separation device.

24. The system of claim 23, wherein said first separation device is selected from  
20 the group consisting of a isoelectric focusing gel electrophoresis device, a free-flow electrophoresis device, a rotofor electrophoresis device, and an ion exchange chromatography device.

25. The system of claim 23, wherein said second separation device is selected from the group consisting of a reversed-phase chromatography device and a hydrophobic interaction chromatography device.

26. The system of claim 23, wherein said third separation device is selected from the group consisting of an SDS-gel electrophoresis device, a size exclusion chromatography  
30 device, and a capillary electrophoresis device.

27. The system of claim 23, where said apparatus further comprises a detection device.

28. The system of claim 27, wherein said detection device is selected from the group consisting of a UV/VS spectrophotometer, a fluorescence spectrophotometer, and a mass spectrometer.

29. The system of claim 28, wherein said mass spectrometer is selected from the group consisting of a MALDI-TOF-MS, a ESI oa TOF, an ion trap mass spectrometer, an ion trap/time-of-flight mass spectrometer; a quadrupole mass spectrometer, a triple quadrupole mass spectrometer, a Fourier Transform (ICR) mass spectrometer, and a magnetic sector mass spectrometer.

30. The system of claim 23, further comprising a protein characterization apparatus in communication with said protein characterization apparatus.

31. The system of claim 30, wherein said protein characterization apparatus is a protein array analysis apparatus.

32. The system of claim 31, wherein said protein array analysis apparatus is configured for performing a functional assay on a separated protein sample.

33. The system of claim 32, wherein said functional assay is an antibody binding assay.